A Technique for the Preparation of Gastropod Chromosomes

BY

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(2 Text figures)

INTRODUCTION

Molluscan chromosomes are receiving increasing attention as their usefulness as another tool in determining systematic relationships is recognized. Many of the cytotaxonomic techniques previously utilized (Husted & Burch, 1946) required elaborate fixing and sectioning procedures, while most of the methods used today employ an acetic-orcein stain fixative as outlined by La Cour (1941). This latter technique has the advantage of being "equally effective with fresh and permanent preparations, the stain is easily regulated and little coloration of the cytoplasm is encountered" (La Cour, *op. cit.*). The technique described below, although partially based upon the techniques outlined by La Cour (*op. cit.*) and McPhail & Jones (1966), details the modifications necessary to adapt their techniques for use with gastropods, expedites the procedure and introduces several new steps.

MATERIALS AND METHODS

The procedure outlined applies to terrestrial pulmonates *ca.* 15 mm in diameter. Using a 26 gauge needle, 0.1 cc (1 mg per ml concentration) of the spindle fiber inhibitor vinblastine sulfate (Eli Lilly & Co., trade name "Velban") is injected directly through the apex of the shell. The specimen is then returned to its container for a period of from 14 to 16 hours. Following this period, beginning at the dorsal lip of the aperture and cutting concentrically toward the apex with scissors, the shell is gradually removed exposing the ovotestis and other soft parts. The ovotestis is dissected out and placed in distilled water for 45 minutes.

The acetic-orcein stain is made up in the following manner: 9 ml of distilled water and 6 ml of glacial acetic acid are poured into a 50 ml capacity Erlenmeyer flask containing 0.1 g orcein, and the resultant mixture is heated to a low boil. The liquid is then filtered through a double layer of filter paper (Whatman No. 1 qualitative filter paper). The gonadal tissue is then placed in the acetic-orcein stain for 30 minutes. After staining, the tissue is lightly rubbed on a microscope slide to produce a slurry of cells. After removing any large pieces of tissue, a cover slip is placed over the smear on top of which is placed the lower, wider half of a number 1 rubber stopper. The entire preparation is then placed on the squashing device, a welder's (spring) clamp bolted to a short section of 5 cm × 10 cm lumber to stabilize it and act as a base (Figure 1), and squashed.

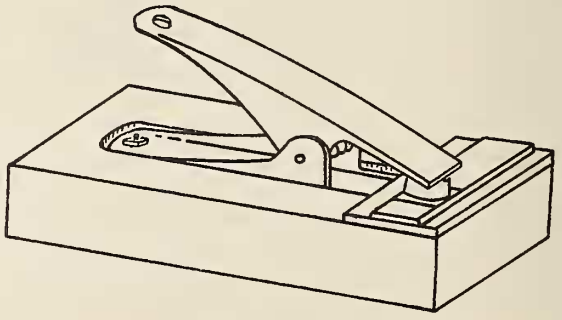

Figure 1

Squashing device, for the preparation of chromosome spreads, showing placement of the slide, cover slip and rubber stopper. A groove is chiseled into the 2"×4" (5 cm×10 cm) block so that the lower blade of the clamp, when bolted into it, is flush with the surface

Temporary preparations can now be made by sealing with Krönig cover glass cement, or another suitable sealant. If kept refrigerated, the preparations may be

preserved in this form for several days. If photographs or sketches are to be made at a later date, permanent preparations can be made in the following way: The slide is placed squarely on dry ice and frozen for about 15 minutes. The cover slip is then removed using a single-edged razor blade. Caution must be exercised here. Inadequate freezing, noted by difficulty in removal of the cover slip and a "sticky" appearance as the cover slip is removed, will result in loss of the spreads. The slide is then immediately immersed in 100% ethyl alcohol for 6 minutes, transferred to a second Coplin jar of 100% ethyl alcohol for an additional 6 minutes and finally to xylene for 6 minutes. If the xylene becomes cloudy, return the slide to 100% ethyl alcohol for 6 more minutes. After semi-drying the slide, Permount and a new cover slip (No. 1) are added. After drying (*ca.* 48 hours) the permanent preparation is ready for reexamination.

DISCUSSION

The technique described above yields many cells in various stages of meiotic activity. It is therefore well suited for determining chromosome numbers.

Recently, Burch (1968) described a tissue culture technique for karyotype analyses of pulmonate land snails and Patterson (1971) presented a karyotype technique using freshwater embryos. As noted by Patterson (*op. cit.*), tissue culture technique is time consuming and requires sterile conditions. Determination of karyotypes and utilization of embryos is also possible with the above described technique, particularly with gravid, viviparous pulmonates and prosobranchs. Utilization of embryos requires the use of a pipette for their removal and transfer from the adult, 16 hours after injection of the "Velban." Squashes are prepared in the manner prescribed above. Stern (1974) using a gravid female prosobranch has obtained mitotic spreads from the actively dividing embryonic cells and mitotic and meiotic spreads from oogonial tissue of the adult.

Preparation of the spreads, following injection and return of the snails to their container, requires only $1\frac{1}{2}$ hours. Using the prescribed manner of dissection, other portions of the soft parts (genitalia, radula, etc.) may also be examined and utilized simultaneously. This is expedient if type specimens are being examined or if only a limited number of individuals is available.

The squashing device described proved especially successful, more so than the use of a vise, or squashing manually. The clamp may be obtained in most hardware stores. The device delivered more than adequate pressure to burst the cells, with the loss of only 2 preparations, out of several hundred, due to breakage.

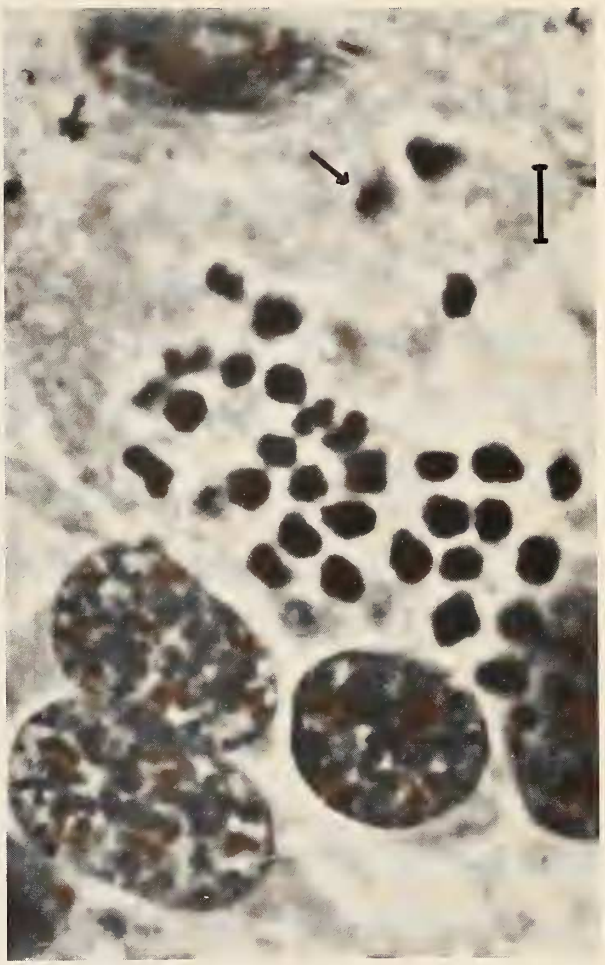

Figure 2
Meiotic chromosomes of *Oreohelix yavapai neomexicana* (n=32) Arrow indicates chromosome which is out of focus; scale line =5μm

Best results have been achieved using adults and "fresh" individuals – *i.e.*, those individuals not recently hibernating or aestivating. Excellent spreads were obtained in 80% of the individuals examined. To date, the technique has been employed only with gastropods.

Meiotic chromosomes of *Oreohelix yavapai neomexicana* Pilsbry, 1905, obtained using this procedure, are seen in Figure 2. The haploid chromosome number is n = 32. This is the first chromosome number reported for a mem-

ber of the stylommatophoran family Oreohelicidae. The "advanced" or "specialized" stylommatophorans have higher chromosome numbers, and the haploid chromosome number (n = 32) obtained for this species is consistent with that of other members of the Sigmurethra, the most advanced of the stylommatophoran suborders.

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Literature Cited

Burch, John Bayard
1968. A tissue culture technique for caryotype analyses of pulmonate land snails. The Venus 27: 20 - 27; 6 figs. (August 1968)

Husted, Ladley & Paul Randolph Burch
1946. The chromosomes of polygyrid snails. Amer. Natural. 80: 410 - 429; 34 figs.

La Cour, L.
1941. Acetic-orcein: a new stain-fixative for chromosomes. Stain Technology 16: 169 - 174; 3 figs.

McPhail, John D. & R. L. Jones
1966. A simple technique for obtaining chromosomes from teleost fishes. Journ. Fish. Res. Board Canada 23: 767 - 769; 2 figs.

Patterson, Charlotte Morgan
1971. A karyotype technique using freshwater snail embryos. Malacol. Rev. 4: 27; 1 fig.

Stern, Edward Marc
1974. The chromosomes of *Viviparus subpurpureus* (Say) (Streptoneura: Viviparidae). In preparation